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Prognostic Impact of Serum Immunoglobulin Heavy/Light Chain Ratio in Patients with Multiple Myeloma in Complete Remission after Autologous Stem Cell Transplantation

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Immunoglobulin heavy/light chain (HLC) ratios were studied in 37 patients with multiple myeloma in complete remission after autologous hematopoietic stem cell transplantation. Increased IgAκ/IgAλ and IgMκ/IgMλ ratios were associated with longer progression-free survival ($P = .006$ and .01, respectively). A statistical trend toward a longer overall survival was also observed for the IgAκ/IgAλ ratio ($P = .068$). Considering the original immunoglobulin isotype, our results indicate that an increased κ/λ ratio of the uninvolved isotype is associated with longer progression-free survival and overall survival. This is the first report demonstrating the association between the HLC ratio and sustained complete remission in patients with multiple myeloma. Our results suggest that the HLC ratio is a surrogate marker of immune recovery after myeloablative transplantation, rather than as a marker of minimal residual disease.

Biol Blood Marrow Transplant 18: 1076-1079 (2012)

KEY WORDS: Residual disease, Isotype, Immune recovery, Plasma cell dyscrasia

INTRODUCTION

Complete remission (CR) in multiple myeloma (MM) is defined by the absence of the original monoclonal protein in serum and urine immunofixations (IFE) [1]. The International Myeloma Working Group proposed the additional category of stringent CR [2], which also requires a normal light chain κ/λ ratio. However, the prognostic significance of this novel category remains under investigation [3,4]. Furthermore, the presence of oligoclonal bands unrelated to the original monoclonal component, which is a frequent event after hematopoietic stem cell transplantation (HSCT) or induction treatments with novel drugs, can result in marked variations in this κ/λ ratio, usually associated with an improved outcome through a robust immune response [5,6].

Automatic assays for the measurement of serum free immunoglobulin chains have been available for a number of years and have proven useful in the screening, diagnosis, prognosis, and follow-up of monoclonal gammopathies [7]. The recent development of antibodies against conjunction epitopes between the light and heavy chains enables the quantitation of specific pairs of heavy/light chains (HLCs: IgGκ/IgGλ, IgAκ/IgAλ, and IgMκ/IgMλ) in serum, which allows the determination of the quantity of tumoral immunoglobulin [8]. The aim of the present study was to establish the possible value of the serum HLC ratios in patients with MM in CR after HSCT.

METHODS

A total of 193 patients with MM underwent autologous HSCT (auto-HSCT) in our institution between March 31, 1994, and December 31, 2010. Of these, 73 achieved CR according to European Blood and Marrow Transplantation (EBMT) criteria. Our analysis included 37 patients (17 males and 20 females; median age, 57 years) who had serum samples available at the time of CR confirmation. All of the patients had achieved CR after melphalan-based auto-HSCT, and

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Financial disclosure: See Acknowledgments on page 1078.

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Received January 17, 2012; accepted March 9, 2012

1083-8791/$36.00
doi:10.1016/j.bbmt.2012.03.004

Table 1. Patient Characteristics

Characteristic	Value
Age, years, median (range)	57 (38-67)
Males/females, n	17/20
Heavy chain type, %	
IgG	51.4
IgA	24.3
Light chains only	13.5
IgD	8.1
IgM	2.7
Light chain type, %	
κ	64.9
λ	35.1
Durie-Salmon stage, %	
I	10.8
II	56.8
III	32.4
International Staging System, %	
1	56.8
2	27
3	16.2
β_2-microglobulin >3.5 mg/dL, %	40.5
Albumin <35 g/L, %	16.2
Lytic bone lesions, %	86.1

had negative serum and urine IFEs for the original monoclonal protein and <5% bone marrow plasma cells [9]. The median duration of follow-up in CR was 4 years (range, 1-17 years), and no patients were lost during follow-up. Relapse and progression were assessed based on EBMT criteria [1]. According to the EBMT criteria, relapse from CR was defined by the confirmed reappearance of the monoclonal band in serum or urine, a >5% increase in plasma cells in a bone marrow aspirate, or the development of hypercalcemia, increased size and/or number of lytic bone lesions, or extramedullary plasmacytomas. Patient characteristics are detailed in Table 1. The main clinical and laboratory characteristics were similar in this group of patients and the remaining patients who achieved CR but who were not analyzed due to lack of sample availability. The study was approved by the Ethics Committee of the Hospital Clínic of Barcelona.

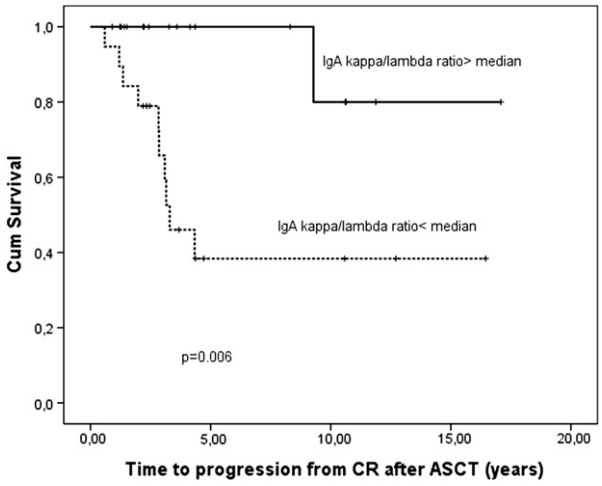

Figure 1. Progression-free survival in the series according to the IgAκ/IgAλ ratio.

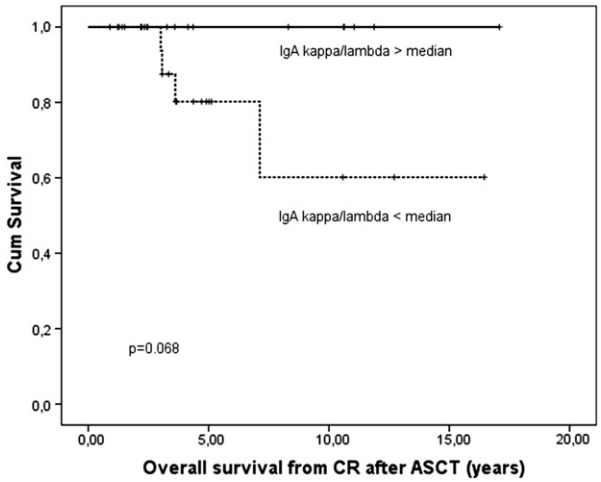

Figure 2. Overall survival in the series according to the IgAκ/IgAλ ratio.

An oligoclonal band was defined as the presence of a heavy and/or light monoclonal immunoglobulin by IFE in serum and/or urine different from the original monoclonal protein [7]. Serum HLC was assayed by immunonephelometry (Hevylite Assay; Binding Site, Birmingham, U.K.), and ratios were determined for each isotype (IgG, IgA, and IgM). Statistical analysis was performed with PASW 18.0 for Windows (IBM, New York, NY). Categorical variables were compared using the χ^2 test or the Fisher exact test, and median differences were compared using analysis of variance or the Student *t* test. Probabilities of overall survival (OS) and progression-free survival (PFS) were estimated with the Kaplan-Maier method and compared using the log-rank test.

RESULTS AND DISCUSSION

Thirty-three of the 37 patients (89.2%) were alive at the time of this analysis; however, 11 (29.7%) had already relapsed from CR. The median PFS and OS from CR were not reached. Induction treatment consisted of bortezomib-based regimens in 15 patients, polychemotherapy in 12 patients, and thalidomide/dexamethasone in 9 patients.

The median values obtained in our patients were not significantly different from the data reported previously in a normal population [8]. Subsequently, we dichotomized our group of patients according to the median value for each pair of HLC isotypes. In our analysis, the IgGκ/IgGλ ratio was not associated with significant differences in PFS or OS. An increased IgAκ/IgAλ ratio was associated with a longer PFS ($P = .006$) and with a statistical trend toward a longer OS ($P = .068$) (Figures 1 and 2). Similarly, an increased IgMκ/IgMλ ratio correlated with a longer PFS ($P = .01$) and with

a trend toward a longer OS ($P = .159$). Regarding the original isotype, neither PFS nor OS was modified in relation to the IgGκ/IgGλ ratio in those patients with an original monoclonal IgG isotype; however, low IgAκ/IgAλ and IgMκ/IgMλ ratios were associated with a shorter PFS ($P = .038$ and .006, respectively). In the patients with an original monoclonal IgA isotype, a lower IgGκ/IgGλ ratio was also associated with shorter PFS ($P = .036$), but no association with the IgA or IgM ratio was observed.

The achievement of CR, with negative serum and urine IFEs, is the most significant prognostic factor in MM [10,11]. Different CR categories are associated with different prognostic impacts. Thus, patients in CR (negative IFEs) without minimal residual disease (MRD) by flow cytometry have a significantly longer PFS and OS compared with those in CR with MRD [12]. In this context, the opportunity to measure HLC ratios provides a new tool for assessing the response to treatment in MM; however, its prognostic utility has not yet been determined.

Our results indicate that increased IgAκ/IgAλ and IgMκ/IgMλ ratios of the uninvolved isotype in patients in CR is associated with longer PFS and OS. Thus, in those patients with MM with an original monoclonal IgG protein who achieved CR, PFS was significantly longer when IgA κ/λ or IgM κ/λ ratios were increased. In this sense, the HLC ratio seems to be a marker of immune reconstitution rather than an indicator of MRD, even though the median values were very close to those reported in the normal population. We found no differences in OS or PFS related to an increased serum immunoglobulin level over the median value for IgA, IgG, or IgM. In fact, we found no correlation between a relatively higher HLC ratio and the corresponding isotype in total immunoglobulins. This represents an additional benefit of measuring HLC ratios over total immunoglobulin levels. Supporting this, the French Myeloma Intergroup reported that the HLC ratio can provide a measure of tumor immunoglobulin production and immunoparesis, and thus would be a useful prognostic factor to add to the current International Staging System [13]. These authors also reported that an abnormal HLC ratio was associated with a shorter PFS independent of β_2-microglobulin and albumin [13]. The Mayo group is also currently investigating the possible value of isotype-specific HLC suppression of uninvolved immunoglobulins as a predictor of progression in monoclonal gammopathy of undetermined significance (MGUS). They reported a series of 30 patients with MGUS who progressed to MM versus 36 patients who did not progress over a similar period of time. Those with MGUS progression had a significantly higher rate of isotype-specific HLC pair suppression than those with stable MGUS [14].

Oligoclonal bands were detected in 64.9% of patients in our series. Of these, 91.7% demonstrated an IgG isotype. No light chain predominance was noted. A second oligoclonal band was found in 29.7% of the cases. In the overall series, no significant relationship between oligoclonal bands and the IgG, IgA, or IgM ratio was detected. Associated with the progressive improvement in CR rates with auto-HSCT, some studies have related the presence of oligoclonal bands (a humoral response unrelated to the baseline M-protein) to better outcome [15]. In a previous study, we reported that 40% of the patients in CR after auto-HSCT presented oligoclonal bands resulting in an elevation in the free light chain (FLC) κ/λ ratio [6]. Increased oligoclonal bands are also seen in patients in CR after immunomodulatory drug therapy [5,16]. In contrast with previous studies that reported modification of the FLC ratio by the presence of oligoclonal bands, there is no evidence that a heavy isotype pair also could be altered by this immune phenomenon. Based on the different nature of the immunoglobulin studied, the meaning of FLC and HLC ratios at diagnosis, prognosis, and follow-up appears to be different. Unlike the FLC ratio, the HLC ratio does not seem to be affected by renal function, given the different excretion pathway of FLCs and HLCs, although there are no specific published data on this. No studies regarding the potential role of active infections or drugs in HLC ratio determination have been reported to date. Similarly, immunoglobulin infusions could potentially modify this value, but this is not the case in the present study, given that none of our patients had immunoglobulin repositioning after transplantation.

In conclusion, our data indicate that a relatively higher HLC ratio of the uninvolved immunoglobulin is predictor for a significantly longer PFS and even OS in patients with MM in CR. Because HLC ratios provide a measure of tumor immunoglobulin production plus immunoparesis rather than a marker of MRD, this parameter is more likely a surrogate marker of robust immune recovery. Further studies investigating potential roles of HLC measurement in MM are warranted.

ACKNOWLEDGMENTS

This work was supported in part by grants RD 2006/0020/0005 and FIS08/0147 from Fondo de Investigaciones Sanitarias de la Seguridad Social and by "Josep Font" and "Emili Letang" grants from Hospital Clínic de Barcelona.

Authorship statement: Natalia Tovar and Carlos Fernández de Larrea contributed equally to this work. Natalia Tovar, Carlos Fernández de Larrea, and Joan Bladé designed the study, collected and

analyzed data, performed statistical analysis, and wrote and reviewed the manuscript. M. Teresa Cibeira and Laura Rosiñol treated the patients, collected data, and reviewed the manuscript. Juan I. Aróstegui, Montserrat Elena, Xavier Filella, and Jordi Yagüe performed the assays and reviewed and approved the manuscript.

Financial disclosure: The authors have no conflicts of interest to disclose.

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